SCU protecting against myocardial injury induced by ischemia reperfusion (IR) are not well known. This study examined whether SCU protects against myocardial IR injury is mediated by the Janus kinase and signal transducer and activator of transcription (JAK/STAT) pathway.
Methods: Three models were used: a rat model of myocardial IR, an isolated thoracic artery (TA) hypoxia reoxygenation (HR) model, and a human cardiac microvascular endothelial cell (HCMEC) HR model. Protein and mRNA expression of JAK2/STAT3 and phosphorylative products were assessed by Western blot, immunohistochemistry and RT-PCR method.
Results: In the rat myocardial IR model, SCU (45 and 90 mg/kg, iv) significantly reduced ischemic size, while immunohistochemical results showed that SCU significantly decreased histological phosphorylation JAK2 (P-JAK2) and STAT3 (P-STAT3) expression. In isolated TA rings, pre-incubation with SCU (100, 500 mmol/L) significantly inhibited JAK2/STAT3 expression after HR. Western blot and RT-PCR of HCMECs indicated that SCU (0.1, 1.0, 10 mmol/L) incubations significantly inhibited the phosphorylation of JAK2 and its downstream molecule STAT3 and in contrast, HR up-regulated them.
Conclusions: SCU attenuates myocardial IR injury, at least in part, by inhibiting injury-induced activation of JAK2/STAT3 signaling pathway.

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Suppression of PKCε-medicated mitochondrial connexin 43 phosphorylation at serine 368 is involved in mitochondrial dysfunction in a rat model of dilated cardiomyopathy

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Objectives: Mitochondrial connexin 43 (Cx43) plays an essential role in ischemic preconditioning cardioprotection, however, it remains unclear whether mitochondrial Cx43 is involved in mitochondrial dysfunction in the pathogenesis of dilated cardiomyopathy (DCM). The present study was performed in order to investigate the changes of the expression and phosphorylation state of mitochondrial Cx43 in a rat model of DCM and to determine whether the altered mitochondrial Cx43 phosphorylation state was involved in mitochondrial dysfunction.
Methods: The rat model of DCM was generated by daily oral administration of furazolidone (FZD) for 30 weeks and was identified by echocardiographic studies. The expression and phosphorylation state of mitochondrial Cx43 were examined by western blot. The expression and activity of protein kinase C (PKC) ε were also analyzed by western blot to reveal the underlying mechanism of Cx43 dephosphorylation at serine 368. And then, the mitochondrial membrane potential level was assessed using JC-1 by quantitative fluorescence measurement. The activities of cytochrome c oxidase and succinate dehydrogenase were determined by quantitative colorimetric assay kit. The primary cultured neonatal rat cardiomyocytes were sparsely plated without cell to cell contact and incubated with 100 nmmol/L phorbol-12-myristate-13-acetate (PMA, a specific PKC activator) for 60 min after 48 h FZD treatment to assess the effects of PKC activation on the FZD-induced mitochondrial Cx43 dephosphorylation and mitochondrial dysfunction. Pretreatment with 18β-glycerrhetinic acid (GA, a connexin channels inhibitor) for 4 h was performed to determine the impact of mtCx43 suppression on PKC-activator induced mitochondrial protection in the FZD-treated cardiomyocytes.
Results: Real-time PCR and western blot revealed the decreased expression of overall Cx43 accompanied with lower level of serine 368-phosphorylated Cx43 immunoreactivity in the myocardium and myocardial mitochondria. Meantime, mitochondrial membrane potential level and the activities of cytochrome c oxidase, succinate dehydrogenase and PKCε were all reduced. PMA partially reversed the FZD-induced mitochondrial Cx43 dephosphorylation serine S368 and the mitochondrial dysfunction in the cardiomyocytes. However, pretreatment with GA abolished the mitochondrial protective effect of PMA in the cardiomyocytes sparsely plated without cell to cell contact.
Conclusions: Our results suggest that mitochondrial Cx43 dephosphorylation at serine 368 due to the suppression of PKCε activity may be a novel mechanism for mitochondrial dysfunction in the pathogenesis of DCM.

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Investigation on Apoptosis of Vascular Endothelial Cells induced by Human Cytomegalovirus via the Fas/FasL Pathway

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Objectives: Human cytomegalovirus (HCMV) infection is associated with cardiovascular diseases, especially atherosclerosis. The detailed mechanisms were not fully understood yet. Vascular endothelial cell injury has been suggested playing a key role in the initiation and progression of atherosclerosis. This study is aiming to determine whether HCMV infection can induce apoptosis in human umbilical vein endothelial cells (HUVEC) via the Fas/FasL pathway, and lead to endothelial cell injury in vitro.
Methods: HUVEC were isolated and cultrured in vitro. DAPI staining method was used to investigate the morphology of apoptotic HUVEC cells, and flow cytometry (FCM) to quantitatively detect the apoptotic rate. An antagonistic anti-Fas antibody was applied to block apoptosis. The transcription of Fas mRNA in the HUVEC cells infected by HCMV AD169 strain and corresponding cells were detected with RT-PCR method. The expression of Fas was detected by FCM using FITC-conjugated monoclonal antibody.
Results: Chromatin condensation and marginalization were found in HUVEC cells infected with HCMV at 48 h and karyorrhexis started presenting in some cells, which became more remarkable at 96 h. The apoptosis rates of HUVEC cells were 21.37% and 55.83% at 48 h and 96 h, respectively, which were decreased to 8.26% and 17.65% after the cells were pretreated with the anti-Fas antibody. The transcriptional level of Fas mRNA in HUVEC cells infected with HCMV AD169 strain showed a strong uptrend over time compared with corresponding cells. Fas expression rates of HUVEC cells rose up to 69.47% and 81.59%, respectively, compared with that of 10.35% and 13.67% in control groups at 48 h and 96 h.
Conclusions: HCMV AD169 strain can up-regulate Fas expression levels of HUVEC cells and induce apoptosis of cells via Fas/FasL pathway. These results suggest that Fas/FasL pathway may play a role in vascular endothelial cell injury and ultimately lead to atherosclerosis.

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Role of GRK4 in the Regulation of Arterial AT1 Receptor in Hypertension

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Objectives: G protein-coupled receptor kinase 4 (GRK4) gene variants, via impairment of renal dopamine receptor and enhancement of renin-angiotensin system functions, cause sodium retention and increase blood pressure. Whether or not GRK4 and the angiotensin type 1 receptor (AT_1R) interact in the aorta is not known.
Methods: GRK4 expression in vascular smooth muscle cells (VSMCs) of the aorta was analyzed by confocal microscopy of double-stained, RT-PCR and immunoblotting. AT1R protein expression and function in GRK4 variant 142V transfected A10 cells and WT cells was quantified by immunoblotting and AT1R-mediated intracellular calcium concentration. AT1R phosphorylation level was determined by immunoprecipitation. The interaction between GRK4 and AT1R was determined by immunoprecipitation and confocal microscopy of double-stained. NF-κB activity was analyzed by electrophoretic mobility shift assay (EMSA). Angiotensin II-mediated vasoconstriction of the aorta from 142V-transgentic mice and WT mcie was analyzed by tension measurement of the artery rings.
Results: We report that GRK4 is expressed in vascular smooth muscle cells (VSMCs) of the aorta. Heterologous expression of the GRK4g variant 142V in A10 cells increased AT_1R protein expression and AT_1R-mediated increase in intracellular calcium concentration. The increase in AT_1R expression was related to an increase in AT_1R mRNA expression via the NF-kB pathway. As compared with control, cells expressing GRK4g 142V had greater NF-kB activity with more NF-kB bound to the AT_1R promoter. The increased AT_1R expression in cells expressing GRK4g 142V was also associated with decreased AT_1R degradation, which may be ascribed to lower AT_1R phosphorylation. There was a direct interaction between GRK4g wild-type (WT) and AT_1R that was decreased by GRK4g 142V. The regulation of AT_1R expression by GRK4g 142V in A10 cells was confirmed in GRK4g 142V transgenic mice; AT_1R expression was higher in the aorta of GRK4g 142V transgenic mice than control GRK4g wild-type (WT) mice. Angiotensin II-mediated vasoconstriction of the aorta was also higher in GRK4g 142V than WT transgenic mice.
Conclusions: This study provides a mechanism by which GRK4, via regulation of arterial AT_1R expression and function, participates in the pathogenesis of conduit vessel abnormalities in hypertension.

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Cardiac deacetylase SIRT3: A mitochondrial target for ischemia reperfusion arrhythmia suppression

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Objectives: Ischemia reperfusion induces a high percentage of lethal arrhythmias. Sirtuin 3 (SIRT3), a key nutrient sensing regulator by governing mitochondrial homeostasis, was also reported to protect heart from reactive oxygen species (ROS) assaults, but whether it is involved in ischemia reperfusion arrhythmias (IRA) and the mechanism underlying remains unknown.
Methods: Sirt3 knockout (SIRT3 KO) mice and littermate wild-type (WT) mice were assigned into sham group, ischemia reperfusion group (I/R) and I/R with NAD treated group (7 days, 1 mg/kg/day) (NAD+I/R). Electrocardiography (ECG) was recorded during I/R for arrhythmia score assessment, and cardiac reactive oxygen species (ROS) production, SIRT3 and MnSOD levels were measured and analyzed.
Results: The results revealed that arrhythmia could be detected in sham SIRT3 KO mice, and more serious arrhythmia was triggered by I/R in SIRT3 KO mice than WT mice ($P<0.05$). Moreover, SIRT3 KO mice showed increased ROS production after I/R compared with WT I/R mice ($P<0.05$), which was in accordance with decreased manganese superoxide dismutase (MnSOD) and catalase (Cat) expression. NAD treatment significantly increased cardiac SIRT3 and MnSOD activity, inhibited ROS production, and consequently suppressed IRA in WT mice, but failed in SIRT3 KO mice.
Conclusions: These findings indicated that impairment of SIRT3 expression with subsequent ROS production played an important role in IRA. Therefore, preserving SIRT3 activity could be a potential approach to prevent IRA.